Stable Expression of Transfected Human Involucrin Gene in Various Cell Types: Evidence for In Situ Cross-Linking by Type I and Type II Transglutaminase

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Involucrin is a 68-kd precursor of the cornified envelope in keratinocytes. During terminal differentiation, involucrin becomes covalently cross-linked to other proteins by a membrane-anchored calcium-activated transglutaminase (TG) to form part of the cornified envelope. To better understand this process, we have used vector-mediated gene transfer to express human involucrin in a variety of cell types. Authentic involucrin protein was expressed in Chinese hamster ovarian (CHO) cells (fibroblasts), PtK2 rat kangaroo kidney cells (simple epithelial), and rat epidermal keratinocytes (stratifying squamous epithelial). The expression vector included an independent transcription unit encoding the aminoglycoside phosphotransferase gene (neo-r) allowing selection of stable involucrin-positive, G418-resistant clonal cell lines. In each cell type, involucrin levels were comparable to the endogenous expression observed in SCC-13 cells. Immunofluorescence localization studies revealed a uniform involucrin distribution throughout the cytoplasm in each cell type. Elevation of intracellular calcium resulted in cross-linking of involucrin in both CHO cells and rat keratinocytes, a process that was inhibited by EDTA and cystamine. In contrast, no cross-linking was observed in PtK2 cells or long-term passaged rat keratinocytes. Transglutaminase assays showed that rat keratinocytes contain predominantly a particulate (type I) form, whereas CHO cells contain only soluble TG (type II). PtK2 cells and long-term passaged keratinocytes, which were unable to cross-link involucrin, contained barely detectable TG activity. These results indicate 1) that involucrin is an efficient in situ substrate for both type I and type II transglutaminase and 2) that involucrin cross-linking is strictly TG dependent. *J Invest Dermatol 97:543–548, 1991*

During the terminal stages of keratinocyte differentiation, most of the intracellular constituents are destroyed by nucleolytic and proteolytic activity [1,2]. In the midst of this destruction of other cellular structures, a new structure, the cornified envelope, is formed beneath the plasma membrane [1,3,4]. The envelope is formed from membrane associated and cytoplasmic constituents and envelope deposition is catalyzed by a specific transglutaminase (TG) that is associated with the plasma membrane [5–8].

Involucrin is an important cytoplasmic precursor of the cornified envelope [2,9]. The central segment of involucrin is composed of a series of tandem linked, highly similar, 10 amino acid repeats [10]. On average, each repeat contains three glutamine residues, each of which is a potential amine acceptor for TG-catalyzed formation of covalent cross-links [3,10]. These cross-links are covalent epsilon-(gamma-glutamyl)lysine isopeptide bonds formed between involucrin and membrane-associated precursors of the cornified envelope during envelope assembly [9,11,12].

As a method of learning more about the function of involucrin, we have used vector-mediated gene transfer to express the involucrin peptide in fibroblasts (Chinese hamster ovarian [CHO] cells), simple epithelial cells (PtK2), and stratified squamous epithelial cells (rat keratinocytes). In each case we have derived clonal cell lines using the gene conferring G418 resistance. In the present report we examine the behavior of the involucrin peptide in each cell type and its ability to participate in the cross-linking reaction.

MATERIALS AND METHODS

Plasmids The involucrin expression plasmid pINVOL-3 was constructed from segments derived from three separate plasmids. Plasmid pECE (a kind gift of Dr. W. Rutter) contains the SV40 early promoter, a polylinker, and the SV40 polyadenylation signal [13]. pSP65Hλ I-3 H6B contains the entire human involucrin gene [10]. Plasmid pREPNEO-Bam$_1$ contains the neo-r gene transcribed using the RSV promoter and SV40 polyadenylation signal plus the Epstein-Barr virus EBNA and ori-P regions in a Bam HI cassette. pREPNEO-Bam$_1$ was derived in our laboratory by extensive modification of plasmid pREP4 [14], kindly provided by Dr. Mark Tykocinski, Case Western Reserve University, Pathology.

The Hinc II/Bam HI segment containing the human involucrin coding sequence and polyadenylation signal from plasmid pSP65 λI-3 H6B was subcloned to Sma I/Bam HI-digested pECE, yielding pECEI-3 Hinc/Bam. The Bam HI fragment including EBNA-1, EBV oriP, and the RSV/neo-r/SV40 stop transcription unit from

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Abbreviations:
DMEM: Dulbecco's modified Eagle's medium
EDTA: ethylenediaminetetraacetic acid
TG: transglutaminase
TRIS: tris[hydroxymethyl]aminomethane

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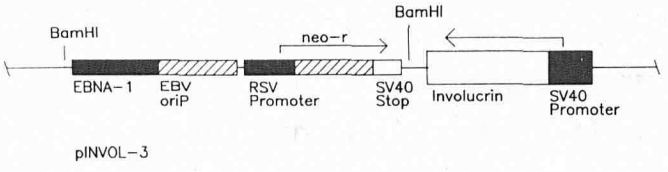

Figure 1. pINVOL-3, the involucrin expression vector. The segments of pINVOL-3 involved in transcription of involucrin and maintenance of the plasmid in eukaryotic cells are shown. The RSV promoter (RSV promoter) and SV40 polyadenylation signal (SV40 stop) regulate transcription of the aminoglycoside phosphotransferase gene (neo-r). Involucrin transcription is initiated at the SV40 promoter/enhancer (SV40 promoter) and terminated at the involucrin polyadenylation sequence located at the left end of the involucrin transcription unit (not shown). EBNA-1 and EBV oriP are elements derived from the Epstein-Barr virus that are responsible for episomal maintenance of plasmids in some cell types [14]. The direction of transcription of the neo-r gene and the involucrin gene relative to the RSV and SV40 promoter, respectively are indicated by arrows. The elements are not necessarily drawn to scale.

pREPNEO-Bam$_1$ was then subcloned into the Bam HI site of pECEI-3 Hinc/Bam to yield plasmid pINVOL-3.

pINVOL-3 includes the human involucrin coding sequence regulated by the SV40 promoter/involucrin polyadenylation signal, the EBNA-1 and EBV oriP sequences and the neo-r gene regulated by the RSV promoter/SV40 polyadenylation signal (Fig 1).

Cell Culture CHO cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% calf serum and hypoxanthine (0.1 mM)/thymidine (0.016 mM). The rat kangaroo kidney epithelial cells (PtK2) and rat epidermal keratinocytes (RAT) were maintained in a 3:1 mixture of DMEM/F12 containing 5 μg/ml insulin, 10 nM hydrocortisone, and 8% fetal calf serum. The rat cell media also contained 5 μg/ml transferrin, 2×10^{-9} M T_3 and 1×10^{-10} M cholera toxin. All media contained 100 U/ml penicillin, 100 mg/ml streptomycin, 50 μg/ml gentamicin, 2 mM L-glutamine, and non-essential amino acids. The rat epidermal keratinocytes are a spontaneously immortalized line kindly provided by Dr. R. Rice (U. California, Davis).

Transfection and Isolation of Cell Lines Cells were seeded at 2×10^5 cells/10 cm dish and transfected as previously described [15]. At two days post-transfection, the cells were split 1:5 and allowed to attach for 24 h. G418 was added at 740 μg/ml (CHO), 300 μg/ml (PtK2), or 125 μg/ml (RAT) and growth was continued until the colonies were large enough for subculture. Individual clones were harvested using cloning rings and transferred to wells in a 24-well cluster plate for growth and later expansion.

Immunofluorescent Detection of Involucrin Cells, grown on glass coverslips, were fixed and permeabilized with methanol/acetone [16] and incubated with preabsorbed rabbit anti-human involucrin antiserum [9]. Specific antibody binding was detected by incubation with a rhodamine-conjugated goat anti-rabbit secondary antibody [16].

Protein Methods and Immunoblotting For detection and quantitation of involucrin in the transfected cell lines, total cell extracts were prepared in Laemmli sample buffer at 5000 cells/μl [17]. Samples (2×10^5 cell equivalents) were fractionated on 8.5% acrylamide denaturing gels [17], transferred to nitrocellulose and incubated with rabbit anti-human involucrin antiserum [9,16]. Specific binding of the primary antibody was visualized using [^{125}I]protein A as previously described [9].

Cross-Linking Assays Near-confluent cultures were harvested with trypsin, washed twice with serum-containing medium and once with serum-free medium, and resuspended in serum-free medium at 5000 cell/μl. Cross-linking was carried out for 4 h at 37°C and was initiated by addition of 0.8 M NaCl to 2×10^5 cells in a final volume of 400 μl. Parallel incubations included 20 mM EDTA as a control to assure that the cross-linking was calcium dependent [5] or 20 mM cystamine to assure that the process was transglutaminase dependent. At the end of the incubation, the cells were pelleted, resuspended at 5000 cells/μl in Laemmli buffer [17] and the extracts (2×10^5 cell equivalents) were fractionated on 8.0% acrylamide denaturing gels. The fractionated proteins were transferred to nitrocellulose and involucrin was detected with rabbit anti-human involucrin primary antibody followed by visualization with [^{125}I]protein A and x-ray film.

Transglutaminase Assay For assay of total TG activity, cells were homogenized at 4°C in TG buffer (10 mM HEPES, pH 7.4, containing 1 mM EDTA, 1 mM dithiothreitol, 10 μg/ml phenyl methyl sulfonyl fluoride, and 1% Triton X-100). For assay of particulate and cytoplasmic forms of TG, cells were homogenized at 4°C in TG buffer lacking Triton X-100 and the extract was centrifuged at 100,000 × g for 30 min (4°C) to yield the cytoplasmic (supernatant) and particulate (pellet) fractions. The pellet was resuspended in TG buffer and extracted for 20 min at 4°C. The total, particulate, and soluble fractions were assayed for TG activity immediately.

The TG assay relies on the ability of TG to transfer [^{3}H]-putrescine to dimethylcasein [5]. The reaction mixture contains 100 mM Tris-HCl, pH 8.2, 0.5 mg of dimethylcasein, 4 mM $CaCl_2$, 5 mM dithiothreitol, 2.5 μCi of [^{3}H]putrescine (10 nmoles), and 20 to 50 μl of extract in a final volume of 250 μl. The mixture is incubated at 37°C for 30 min and formation of [^{3}H]putrescine-dimethylcasein conjugates is monitored by precipitation with 10% trichloroacetic acid followed by scintillation counting [5]. The results are expressed as pmoles [^{3}H]putrescine/mg protein.

RESULTS

The cell lines utilized in the present study are described in Table I. They include CHO cells (fibroblast), rat kangaroo kidney epithelial cells (simple epithelial), and the rat epidermal keratinocytes (stratified squamous epithelial). These cell types were selected because they represent a range of cell lineages, each of which has a different morphology and function. In addition, none of these cell types express any proteins that react with anti-human involucrin antibody. Although rat keratinocytes may express an involucrin-like molecule, it is not detected with the anti-human involucrin antibody. Involucrin is exclusively expressed in stratifying squamous epithelia in vivo [9]. Thus, involucrin expression in other cell types, each containing different components of the cross-linking system, may provide insights into the cross-linking mechanism.

Involucrin expression was accomplished using the expression vector, pINVOL-3 (Fig 1). Cell lines were transfected with 10 μg of DNA and after 72 h G418 was added to the medium. The transfection efficiency averaged 0.01 to 0.05%, as measured by outgrowth of G418 resistant colonies. Colonies were subsequently cloned and tested for involucrin expression. Essentially all of the G418 selected cell lines expressed involucrin. Approximately 20 to 40 clones were selected for each cell type. Three involucrin expressing cell lines are described in the present study; CHO-I (line 8A), PtK2-I (line 12D), and RAT-I (line IE-1.C1).

Involucrin is expressed at a level of 0.1% of total cell protein in cultured epidermal keratinocytes [9] and cultured ectocervical epithelial cells [16]. To evaluate how much involucrin was being expressed in our cell lines, we prepared extracts from CHO-I, PtK2-I, and RAT-I cells and from the corresponding parent cell lines, and screened for involucrin expression by immunoblotting whole cell

Table I. Cell Lines Used in Expression Studies

Cell Line	Description	Filament Type
CHO	Chinese hamster ovarian (mesenchymal, fibroblast)	Vimentin
PtK2	Rat kangaroo kidney epithelial (simple epithelial)	Keratin
Rat-Ker	Rat epidermal keratinocyte (stratifying squamous)	Keratin

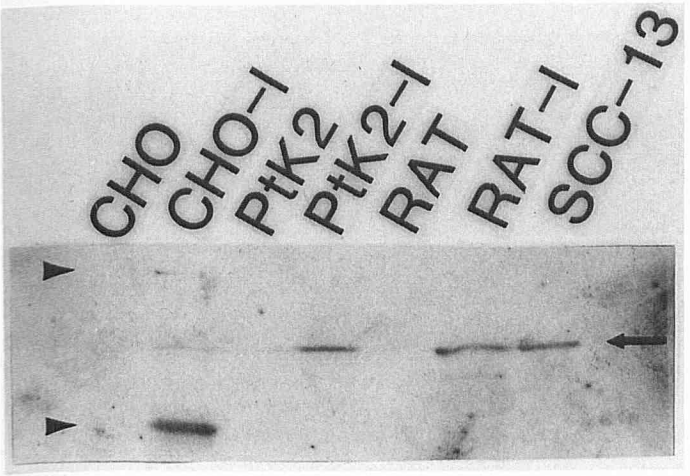

Figure 2. Stable expression of involucrin in CHO, PtK2, and rat epidermal keratinocytes. CHO, PtK2, and rat cells were transfected with pINVOL-3 and clonal cell lines were selected using G418. Extracts were prepared from nontransfected (CHO, PtK2, RAT) and transfected (CHO-I, PtK2-I, RAT-I) cells and equal cell equivalents were electrophoresed on an 8% acrylamide gel, transfer blotted to nitrocellulose, and screened with involucrin-specific antibody. SCC-13 extract was included as a marker for the migration of authentic involucrin. *Arrow,* position of authentic involucrin *(right); arrowheads,* migration of two other immunoreactive bands observed in some of the CHO clonal cell lines.

extracts using an involucrin-specific antiserum. Figure 2 shows that extracts from untransfected cells contain no immunoreactive proteins, whereas extracts from the transfected cell lines contain an immunoreactive molecular that comigrates with authentic involucrin. The level of involucrin expression in each of the cell lines is comparable to that observed in SCC-13 cells, a human squamous cell carcinoma cell line that expresses involucrin at one tenth the level of normal human keratinocytes. The CHO-I cells expressed somewhat more involucrin than PtK2-I and RAT-I cells. As shown in Fig 2, immunoreactive proteins in CHO-I total cell extracts migrated at three distinct positions; one form co-migrates with authentic involucrin, whereas the other two forms display faster and slower mobilities *(arrowheads)*. In this sample, the faster migrating form is more abundant; however, the relative ratio of these forms varies between experiments. At the present time, the etiology of these forms is unknown. The PtK2-I and CHO-I cells express a single immunoreactive band migrating with authentic involucrin *(arrow)*.

Immunofluorescence studies of normal human keratinocytes and ectocervical epithelial cells demonstrate that involucrin has a uniform cytoplasmic distribution [16,18]. We therefore examined whether it behaved in a similar manner in the transfected cell lines. Cells, grown on glass coverslips, were fixed and incubated with anti-involucrin antibody followed by rhodamine-conjugated second antibody. As shown in Fig 3, involucrin displayed a uniform cytoplasmic distribution in all transfected cell types, similar to its distribution in SCC-13 cells. In addition, although these are clonal cell lines, the level of involucrin expression varied from cell to cell.

During terminal keratinocyte differentiation, involucrin is cross-linked and incorporated into the cornified envelope by a specific, calcium-dependent TG [5,12,19]. A standard method of determining whether involucrin becomes cross-linked is by making tissue culture cells permeable to calcium using ionophores or NaCl and then monitoring for the loss of involucrin from the soluble phase [9,11]. To determine whether the transfected cell lines could incorporate involucrin into an insoluble structure, we elevated intracellular calcium by permeabilizing the cells with 0.8 M NaCl. Whole cell extracts were prepared and the level of soluble involucrin assayed by immunoblotting. In the RAT-I keratinocytes, involucrin disappeared from the soluble fraction in the presence of NaCl (Fig 4, *lane 2*). This loss was inhibited by addition of the calcium chelator, EDTA *(lane 3)* or the TG inhibitor, cystamine *(lane 4)*. Similarly, involucrin also disappeared from the soluble fraction in CHO-I cells *(lane 2)* and its loss was inhibited by EDTA *(lane 3)* or cystamine *(lane 4)*. In contrast to the results with RAT-I and CHO-I cells, no involucrin loss from the soluble fraction was observed in PtK2 cells (Fig 5). These results suggest that involucrin is a substrate for CHO and rat keratinocyte TG, but not for PtK2 TG.

In keratinocytes, the TG that catalyzes envelope formation is a particulate enzyme [5,9]; therefore, we assayed CHO-I, PtK2-I, RAT-I cells and normal keratinocytes to determine the level and subcellular distribution of the endogenous TG activity. Normal human keratinocytes were selected as the control cell type for the TG experiments because they express high levels of TG activity. The total TG activity profile of each cell type is shown in Fig 6 (the percent of the activity found in the particulate fraction is indicated at the top of each bar). RAT-I cells and normal human keratinocytes express similar amounts of TG activity, CHO cells express less, and PtK2 cells express barely detectable activity. Ninety percent of the activity in the RAT-I cell line and in normal human keratinocytes (KER) is particulate, whereas less than 1% is particulate in CHO-I cells. It is interesting that involucrin is cross-linked in the two cell types that express appreciable amounts of TG, regardless of whether the TG is soluble or particulate, but not in the TG-negative PtK2 cells.

In the course of these studies, we discovered that prolonged passage of the Rat-I cells results in continuous stable expression of the transfected involucrin gene, but a complete loss of TG activity. When these TG-negative Rat-I cells are challenged with NaCl, involucrin no longer disappears from the soluble phase, strongly suggesting that its disappearance in the early passage RAT-I cultures is due to TG-mediated cross-linking.

DISCUSSION

Involucrin is an important envelope precursor that is incorporated into the cornified envelope during terminal keratinocyte differentiation [1,2]. Its properties, a long axial ratio [9] and regularly-spaced, multiple amine acceptor sites [3,10], suggest that it is likely to be an important peptide cross-linker in the envelope [20]. The cross-links are formed between the gamma-glutamyl groups of involucrin and primary amines donated by other cellular proteins [21]. Bond formation is catalyzed by a keratinocyte-specific membrane-bound TG [5,19]. The involucrin amino acid sequence, derived from the DNA coding sequence [10], includes a central segment of 39 highly similar repeats of 10 amino acids each. Each repeat contains, on average, three glutamine residues. Each of these glutamines are potential in vivo cross-linking sites, although a recent report suggests that glutamine 496 is preferred in vitro [22].

Previous studies indicate that cross-linked envelope formation can proceed in vitro in whole cell extracts [12,22]. Although cross-linking was observed in extracts from both keratinocytes and fibroblasts (3T3 cells), the cross-linking appeared to be less efficient in the fibroblast extracts. In vitro cross-linking experiments have two inherent problems. First, cross-linking is likely to be less efficient in vitro than in intact cells and second, subcellular compartmentalization, which may be important in cross-linking, is lost.

To circumvent these problems, we have expressed the involucrin protein in a variety of cell types and then monitored its ability to participate in cross-link formation. CHO cells (fibroblasts), PtK2 cells (simple epithelial cells), and rat keratinocytes (stratifying squamous epithelial cells) were selected for this study because they represent different cell lineages (fibroblasts, simple epithelial, and stratifying squamous epithelial) and because they have different TG types. It should be noted that rat keratinocytes most likely possess an involucrin-like molecule; however, it cannot be detected using the anti-human involucrin antibody. When expressed using the SV40 promoter element, involucrin levels in the transfected cell lines are comparable to the levels found in SCC-13 cells, approximately one

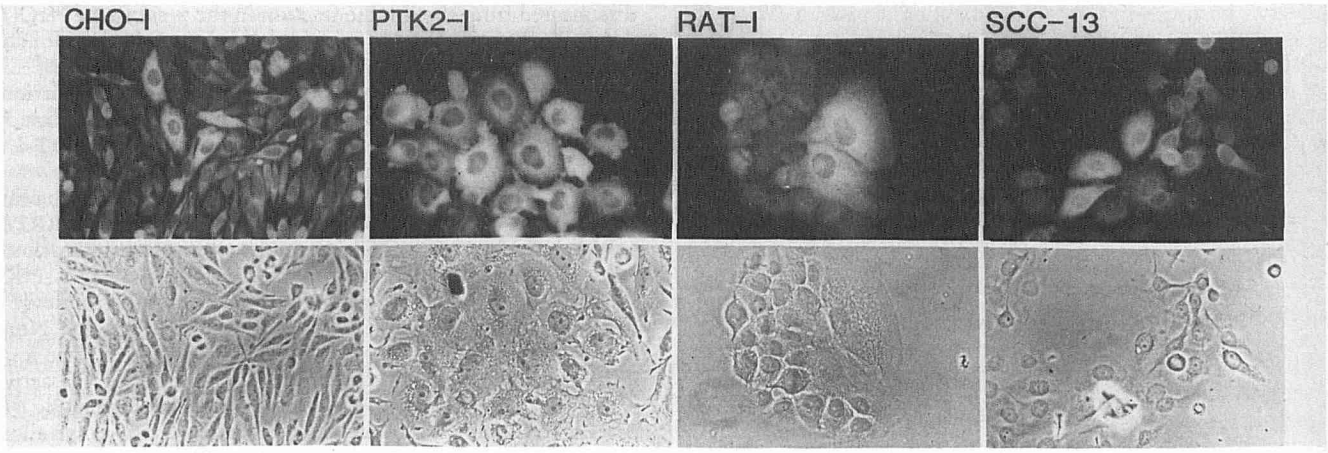

Figure 3. Intracellular distribution of involucrin. The cell lines described in Fig 2 were grown on glass coverslips, fixed with methanol/acetone, and incubated with involucrin-specific antibody followed by incubation with rhodamine-conjugated goat anti-rabbit second antibody. CHO-I, PtK2-I, and RAT-I are clonal cell lines expressing human involucrin. SCC-13 cells are a human squamous cell carcinoma cell line [34] that express involucrin as an endogenous product. The *bottom panels* show the phase-contrast images. The *upper panels* show the corresponding immunofluorescent images of the identical field.

tenth the level present in normal keratinocytes. In fact, more involucrin is expressed in the transfected CHO-I cells than in SCC-13. Interestingly, in each cell type tested, involucrin displayed a cytoplasmic localization that was indistinguishable from that seen in SCC-13 cells (Fig 3), normal keratinocytes [18], and normal human ectocervical cells [16]. Thus, the cytoplasmic compartmentalization appears similar whether the cell is a fibroblast, simple epithelial cell or stratifying squamous epithelial cell. Although each cell line was clonally derived, involucrin expression within individual cells was highly variable (Fig 3), similar to the uneven endogenous expression observed in SCC-13 cells (Fig 3). This was somewhat surprising, because involucrin expression in the transfected cell lines is

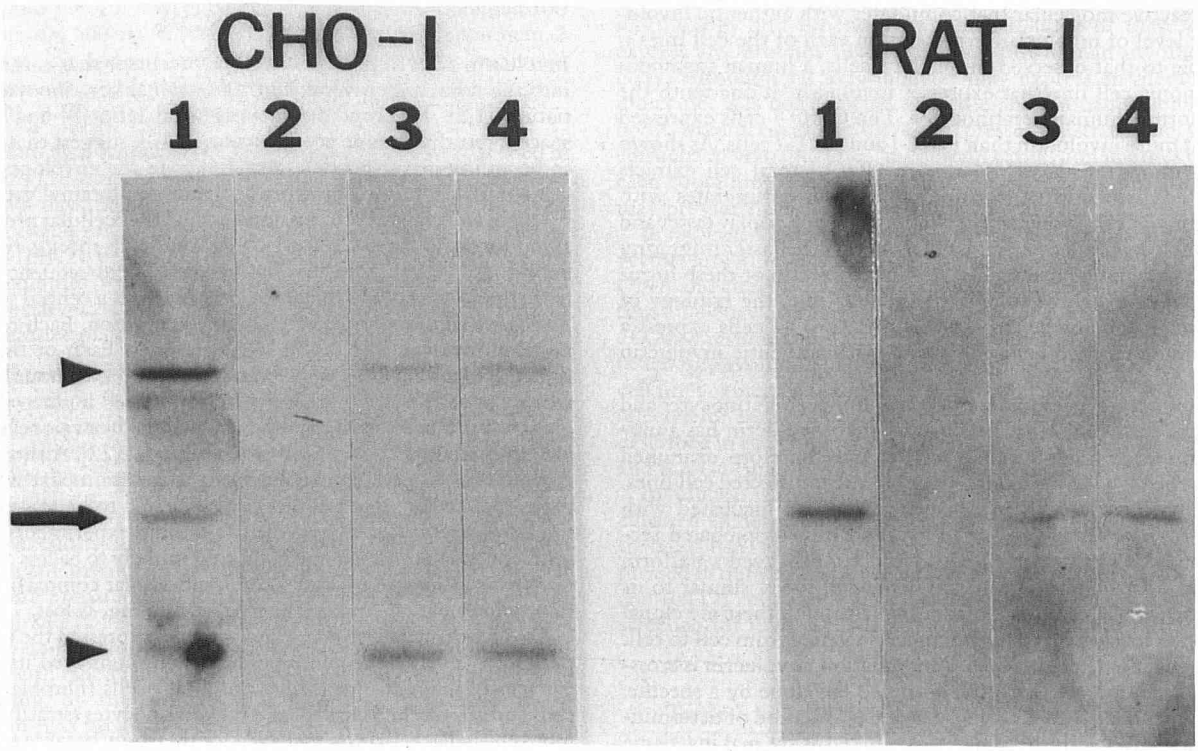

Figure 4. Involucrin cross-linking in CHO-I and RAT-I cells. CHO-I and RAT-I cells were harvested and resuspended in serum-free, 1.5 mM calcium-containing medium. Transglutaminase was activated by permeabilizing the cells with 0.8 M NaCl. After 4 h at 37°C the cells were collected, resuspended in Laemmli buffer [17], fractionated electrophoretically, and transferred to nitrocellulose. Involucrin was detected using an involucrin-specific antibody and [^{125}I]protein A [16]. *Lane 1,* control (no NaCl, EDTA or cystamine); *lane 2,* 0.8 M NaCl; *lane 3,* 0.8 M NaCl + 20 mM EDTA; *lane 4,* 0.8 M NaCl + 20 mM cystamine. *Arrow,* migration of authentic involucrin; *arrowheads,* forms that are observed only in CHO-I. Similar results were derived in each of five separate experiments.

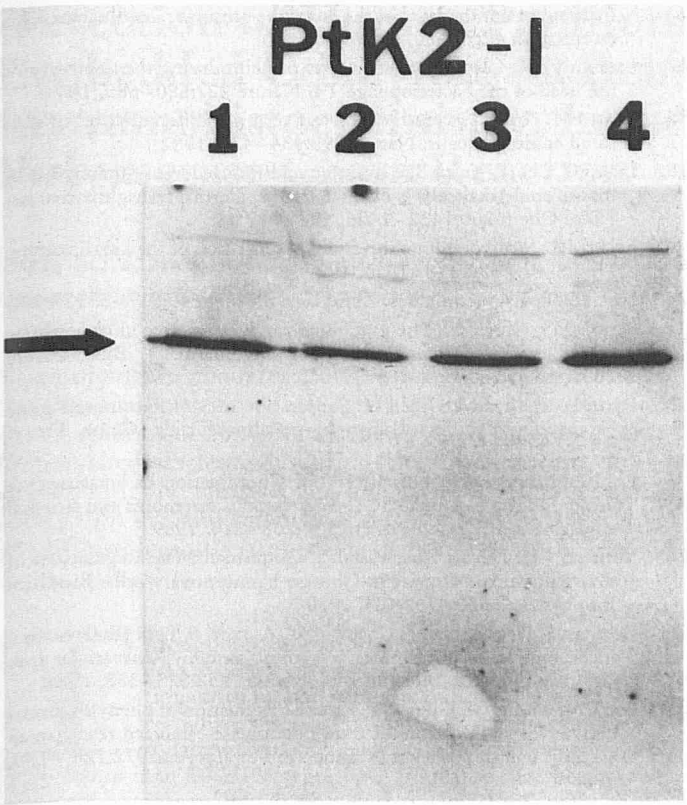

Figure 5. Lack of involucrin cross-linking in PtK2-I cells. The experiment was performed exactly as described in Fig 4. *Lane 1,* control; *lane 2,* 0.8 M NaCl; *lane 3,* 0.8 M NaCl + 20 mM EDTA; *lane 4,* 0.8 M NaCl + 20 mM cystamine. *Arrow,* migration of authentic involucrin. Similar results were observed in each of four experiments.

driven by the SV40 promoter. This may suggest that the activity of the SV40 promoter is regulated as a function of cell cycle or differentiation [23], or that involucrin message or protein stability varies during the cell cycle. At present we have not distinguished among these alternatives.

Transglutaminases are a family of calcium-dependent enzymes that catalyze epsilon-(gamma-glutamyl)lysine bond formation [21]. Several types of TG have been identified, including factor XIIIa and type I and type II enzymes [21]. Factor XIIIa is localized in both the intracellular and extracellular compartments, whereas the type I and type II enzymes are intracellular. The type II (tissue) TG has a ubiquitous distribution, whereas the type I (epidermal) TG is restricted to stratifying epithelial cells [5,21,24].

Elevation of intracellular calcium resulted in cross-linking of involucrin in CHO-I and RAT-I cells, the two cell types that expressed appreciable TG activity. The cross-linking was inhibited by chelating agents and cystamine, indicating the requirement for TG activity. No cross-linking was observed in PtK2 cells, which express barely detectable TG. Moreover, after prolonged passage, RAT-I cells undergo dedifferentiation and suffer a complete loss of TG activity; these TG-negative cells also fail to cross-link involucrin.

As verified in the present experiments, several previous reports indicate that CHO cells express the soluble type II (tissue type) TG that is able to cross-link CHO cellular proteins to form higher molecular weight aggregates [25–27]. Rat keratinocytes express the type I, epidermal cell-specific, TG [28] (Fig 6). Our results suggest that involucrin is a substrate for both tissue type and keratinocyte-specific TG and that cross-linking in situ does not occur in the absence of transglutaminase activity. Taken together these results indicate that involucrin is not likely to be a highly specific substrate for any particular type of TG, but that it can interact with many or all forms. This idea is consistent with the literature on known substrates for TG, because no common amino acid sequence has been identified that defines a TG substrate region [20,29–33]. In fact, it has been suggested that the target sites for cross-linking by TG may be defined by the secondary structure of the substrates [22,33].

Previous in vitro experiments demonstrated that extracts from keratinocytes (type I TG) and 3T3 cells (type II TG) could cross-link involucrin, although the 3T3 extracts were considerably less efficient [12]. TG activity expressed as pmoles/mg protein is 3 times lower in CHO-I cells compared to RAT-I cells, yet involucrin disappears completely from the soluble phase during the course of our assay in both cell types (Fig 4). This suggests that involucrin is an efficient substrate for the type II TG expressed in CHO cells.

In the present experiments we could not identify involucrin in insoluble or high molecular weight structures following cross-linking. Because involucrin probably becomes cross-linked to a variety of different amine donors making it difficult to detect after cross-linking, its immunoreactive epitopes may be shielded following cross-linking. This is similar to the situation in vivo, where involucrin immunoreactivity is lost in the upper layers of stratum corneum following its incorporation into the cross-linked envelope. A second possibility is that involucrin is not being cross-linked, but rather is being digested by proteases. We consider this unlikely for several reasons: cross-linking is inhibited by chelation of calcium, a necessary co-factor for TG function or by addition of cystamine, a specific inhibitor of TG activity. It seems unlikely that the EDTA and cystamine, two agents that inhibit TG by completely different mechanisms, could both act to inhibit a common involucrin-degrading protease. Finally, involucrin disappearance (cross-linking) is strictly correlated with the presence of measurable TG activity, because PtK2-I cells and long-passaged RAT-I cells that lack TG activity do not cross-link involucrin.

In summary, our results indicate that 1) involucrin assumes a keratinocyte-like cytoplasmic distribution when expressed in fibroblasts, simple epithelial cells and stratifying squamous epithelial cells; 2) involucrin is a reasonably efficient substrate with respect to its interaction with type I and type II TG; and 3) involucrin cross-linking is strictly TG dependent in each cell type tested.

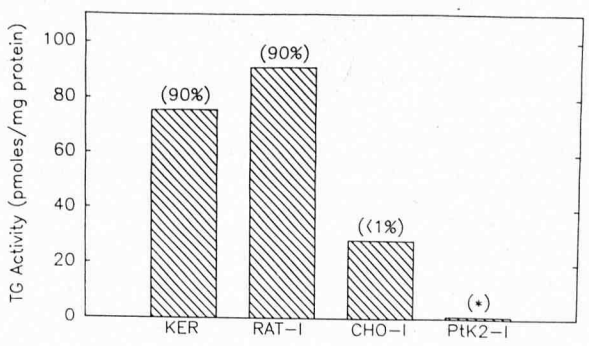

Figure 6. Transglutaminase activity in transfected cell lines. Four day post-confluent cultures of CHO-I cells, PtK2-I cells, RAT-I cells, and normal human keratinocytes (KER) were harvested by scraping and assayed for total and particulate bound transglutaminase activity. *Bars,* total TG activity expressed as pmoles [^{3}H]putrescine/mg protein. *Numbers in parentheses,* percent of TG activity associated with the particulate fraction. The *asterisk* over PtK2-I indicates that the particulate level could not be accurately determined, because the overall activity was barely above background. Similar results were observed in three separate experiments. The low activity present in the particulate fraction in CHO-I cells most likely represents contamination of the particulate fraction with soluble enzyme.

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